Drosophila Eye Model to Investigate how mutations in OPA1 and Drp1 genes contribute to neurodegeneration in Alzheimer's Disease

Elizabeth Borchers[1,2], Lydia Payton[1,2], Neha Gogia[1], Ankita Sarkar[1]
Amit Singh[1,2,3,4]

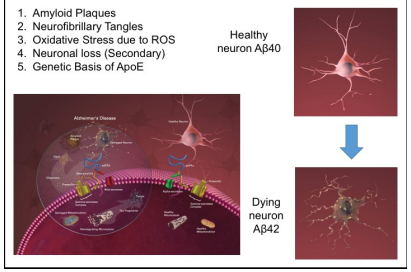
1) Department of Biology University of Dayton, 300 College Park Drive, Dayton, OH; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, 300 College Park Drive, Dayton, OH; 4) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN, USA.

Abstract

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder with no known cure to date. This disease is caused by the extra-cellular accumulation of amyloid-beta 42 (Aβ42) peptides, which results in neuronal death. We have developed transgenic *Drosophila melanogaster* (a.k.a fruit fly) model of AD where human Aβ42 peptides are misexpressed using the GAL4/UAS system specifically in the *Drosophila* eye. Our model organism, *Drosophila*, is used because of its ability to reproduce quickly, and its genome is highly conserved with humans. The GAL4/UAS system allows Aβ42 accumulation only in the differentiating photoreceptor neurons, which kills only the retinal neurons, but does not affect the reproductive ability as well as life span of flies. One of the hallmarks of AD is generation of reactive oxygen species (ROS) from mitochondria, which triggers neuronal death. We hypothesize that OPA1, and Drp1, (dynamin related GTPases) which regulate mitochondrial fusion and fission respectively are involved in regulating Aβ42 mediated neurodegeneration. A fine balance between mitochondrial fission and fusion events is essential for normal mitochondrial and cellular function. Mutations of OPA1 (an early stop signal) produce small unstable mitochondrial proteins, which increase ROS levels in neurons. There is a strong correlation between increased ROS levels and mitochondrial fragmentation with neuronal death. Here, we investigate the role of both OPA1 and Drp-1 in Aβ42 mediated neurodegeneration using *Drosophila* as our model. These genes are highly conserved between flies and humans, so information generated can be extrapolated into humans. Our results show that up-regulating OPA1 produced an eye rescue (reverse in neurodegeneration) in only female flies, while down-regulating OPA1 produced an eye rescue in only male flies. Down-regulating Drp1 produced an eye rescue in only female flies, while up-regulating Drp1 did not produce any rescues. More research on how mitochondrial maternal inheritance will help us better understand these results.

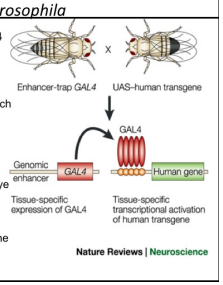
Alzheimer's Disease

1. Amyloid Plaques
2. Neurofibrillary Tangles
3. Oxidative Stress due to ROS
4. Neuronal loss (Secondary)
5. Genetic Basis of ApoE



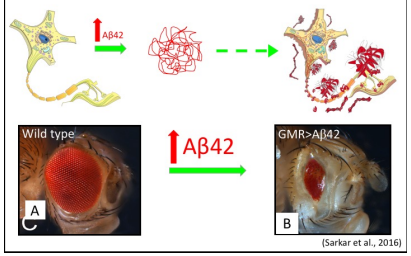
Target gene expression using Gal4 UAS system in *Drosophila*

- GAL4 sequence codes for GAL4 protein which is attached to a tissue specific promoter.
- GAL4 binds to Upstream activating sequences (UAS) which is attached to our gene/s of interest (ex. human Aβ42).
- Transcription of Aβ42 gets triggered specifically in the photoreceptor neurons (in the eye region.)
- The resultant flies are screened on the basis of phenotypes in the eye.



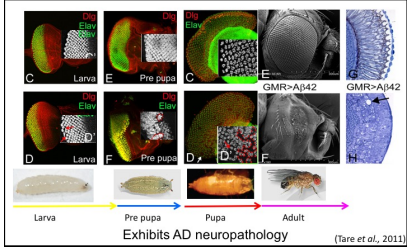
(Brand and Perrimon, 1993)

Our Fly eye model exhibits neurodegenerative phenotype seen in AD



(Sarkar et al., 2016)

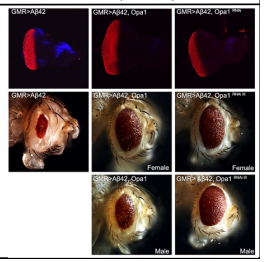
Misexpression of Aβ42 in eye exhibits progressive neurodegenerative phenotype



Exhibits AD neuropathology (Tare *et al.*, 2011)

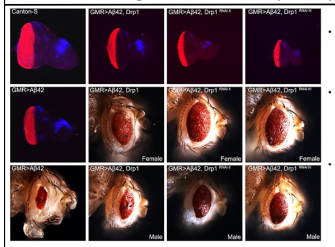
Downregulating Opa1 Levels rescue Aβ42 mediated neurodegeneration in *Drosophila* eye

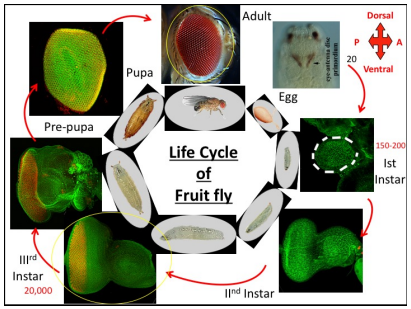
- GMR> Aβ42 shows neurodegeneration
- GMR> Aβ42, Opa1 eye rescue in only females
- GMR> Aβ42, Opa1$^{RNAi\ III}$ eye rescue in only males



Downregulating Drp1 Levels rescue Aβ42 mediated neurodegeneration in *Drosophila* eye

- GMR> Aβ42, Drp1 shows neurodegeneration
- GMR> Aβ42, Drp1$^{RNAi\ II}$ eye rescue in only females
- GMR> Aβ42, Drp1$^{RNAi\ III}$ eye rescue in only females





Results, Conclusions and Future Directions

Results -

- GMR> Aβ42, Opa1 eye rescue in only females.
- GMR> Aβ42, Opa1$^{RNAi\ III}$ eye rescue in only males.
- GMR> Aβ42, Drp1$^{RNAi\ II}$ eye rescue in only females.
- GMR> Aβ42, Drp1$^{RNAi\ III}$ eye rescue in only females.

Conclusions -

- Our transgenic model exhibits AD like neuropathology.
- Fusion and fission are critical for the survival of the cell since mitochondria's role is the production of ATP.

Future Directions -

- Further investigation of the role of OPA1 and Drp1 in Aβ42 mediated neurodegeneration in Alzheimer's Disease.
- Further investigation of the role of mitochondrial maternal inheritance.

Acknowledgement: We would like to thank the Bloomington Stock Center & DSHB for the reagents. EB is supported in part by the Premedical Program, University of Dayton. AS is supported by NIH 1R15GM124654-01 Grant and STEM Catalyst Grant. AS is supported by Department of Biology, University of Dayton. NG is supported in part by the University of Dayton Office for Graduate Academic Affairs through the Graduate Student Summer Fellowship Program (GSSF) and the Department of Biology, University of Dayton.